Int J Endocrinol Metab. 2020 October; 18(4):e108510. doi: 10.5812/ijem.108510.

Published online 2020 October 19. **Research Article**

NOL4 is Downregulated and Hyper-Methylated in Papillary Thyroid Carcinoma Suggesting Its Role as a Tumor Suppressor Gene

Sara Sheikholeslami [1], Fereidoun Azizi [2], Asghar Ghasemi [3], Abbas Alibakhshi [4], Hossein Parsa [5], Seyed Mohammad Tavangar [6], Setareh Shivaee [1], Marjan Zarif Yeganeh [1], Mehdi Hedayati [1,*] and Ladan Teimoori-Toolabi [7,**]

[1]Cellular and Molecular Endocrine Research Center, Research Institute for Endocrine Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[2]Endocrine Research Center, Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[3]Endocrine Physiology Research Center, Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[4]Department of General Surgery, Imam Khomeini Hospital Complex, Tehran University of Medical Sciences, Tehran, Iran
[5]Department of Surgery, Velayat Hospital, Qazvin University of Medical Sciences, Qazvin, Iran
[6]Department of Pathology, Dr. Shariati Hospital, Tehran University of Medical Sciences, Tehran, Iran
[7]Molecular Medicine Department, Biotechnology Research Center, Pasteur Institute of Iran, Tehran, Iran

[*]*Corresponding author*: Cellular and Molecular Endocrine Research Center, Research Institute for Endocrine Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran. Email: hedayati@endocrine.ac.ir
[**]*Corresponding author*: Molecular Medicine Department, Biotechnology Research Center, Pasteur Institute of Iran, Tehran, Iran. Email: lteimoori@pasteur.ac.ir

Received 2020 August 15; **Revised** 2020 September 08; **Accepted** 2020 September 19.

Abstract

Background: Thyroid cancer is the fourth most common cancer in the world. Papillary thyroid carcinoma (PTC) accounts for 80% of all types of thyroid neoplasm. Epigenetic alterations such as DNA methylation are known as the main cause of different types of cancers through inactivation of tumor suppressor genes.
Objectives: In the present study, the expression and methylation of suggested gene namely nucleolar protein 4 (*NOL4*) in PTC in comparison to multi nodular goiter (MNG) have been studied.
Methods: Forty-one patients with PTC and 38 patients affected by MNG were recruited. Thyroid tissues were obtained during thyroidectomy. RNA and DNA were extracted from thyroid tissues. Quantitative RT-PCR assay was performed for determining the mRNA level of *NOL4* while methylation-sensitive high resolution methylation was applied for assessing the methylation status with designing six pairs primers for six regions on gene promoter which were named from NOL4 (a) to NOL4 (f).
Results: Methylation assessment of 81 CpG islands in the promoter region of *NOL4* gene revealed that *NOL4* (f), the nearest region to the start codon, was significantly hypermethylated in PTC cases compared to MNG cases. *NOL4* level in PTC cases in comparison with MNG cases were downregulated. The methylation status and mRNA level of *NOL4* (f) were associated with age of diagnosis (Age of the patient at the time of diagnosis), lymph node metastasis, and advanced stages of disease.
Conclusions: These data suggested an aberrant promoter hyper-methylation of *NOL4* in PTC cases may be linked with its downregulation. Therefore, *NOL4* gene can be proposed as a potential tumor suppressor gene in PTC tissues.

Keywords: DNA Methylation, Gene Expression, Genes, Tumor Suppressor, *NOL4*, Thyroid Cancer, Papillary

1. Background

Thyroid cancer is the fourth most common cancer worldwide. Its incidence has increased over the last decades. Based on the Surveillance, Epidemiology, and End Results program (SEER) data, the number of new cases of thyroid cancer were 52,070 and the percentage of all new cancer cases were 3% in 2019 (http://seer.cancer.gov/statfacts/html/thyro.html). This rate will increase to 52,890 new cases at the end of 2020 (1). Iran Cancer Data System Registry has reported 10,913 cases of thyroid cancer (7 - 90 years old) while the total incidence rate (per one year) was 2.20 per 100,000, during 2004 - 2010 (2). These findings show the increased rate of thyroid cancer in recent years. Thyroid carcinoma is classified into four groups; papillary thyroid carcinoma, follicular thyroid carcinoma, medullary thyroid carcinoma and anaplastic type. papillary thyroid carcinoma is the most common type of thyroid cancer accounting for about 80% of thyroid neoplasms (3, 4).

B-Raf proto-oncogene serine/threonine kinase (*BRAF*) mutations, especially V600E, are the most important genetic factors, being responsible for different aggressive phenotypes such as angiogenesis and tumor invasion (5).

Copyright © 2020, International Journal of Endocrinology and Metabolism. This is an open-access article distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (http://creativecommons.org/licenses/by-nc/4.0/) which permits copy and redistribute the material just in noncommercial usages, provided the original work is properly cited.